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Abstract: Ecosystem stability is threatened by multiple global change factors such as drought and elevated nitrogen deposition. Yet, it is still poorly understood whether soil organisms can buffer against such perturbations. Here we focus on arbuscular mycorrhizal fungi (AMF), a common and widespread group of soil fungi. AMF form symbiotic associations with the majority of terrestrial land plants and promote a range of ecosystem services including plant production, diversity and nutrient cycling. We tested whether AMF have the ability to enhance the resistance and resilience of plant communities under soil moisture deficit (hereby drought) and nitrogen deposition. Grassland microcosms with 11 different plant species were established with and without AMF and exposed to elevated nitrogen levels and to an intermittent period of drought. Drought strongly reduced plant productivity and nitrogen cycling, but had limited effects on plant diversity. Nitrogen enrichment reduced plant diversity and increased nitrogen leaching and N2O emissions. The presence of AMF enhanced plant productivity, plant diversity and reduced nitrogen losses. AMF facilitated the resistance of plant productivity and nitrogen cycling to drought and the recovery of the plant community structure back to its pre drought state. Furthermore, AMF also mitigated the adverse effects of nitrogen enrichment on the resistance of multiple ecosystem functions to drought. Synthesis. Our work highlights the integral role of AMF for the stability of ecosystem functioning; AMF are not only able to promote resistance to harsh conditions of global change but also improve resilience by enabling plant communities to recover. These findings underline AMF's insurance capacity to buffer ecosystems against global change.

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Symbiotic soil fungi enhance resistance and resilience of an experimental grassland to drought and nitrogen deposition

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Abstract

1. Ecosystem stability is threatened by multiple global change factors such as drought and elevated nitrogen deposition. Yet, it is still poorly understood whether soil organisms can buffer against such perturbations. Here we focus on arbuscular mycorrhizal fungi (AMF), a common and widespread group of soil fungi. AMF form symbiotic associations with the majority of terrestrial land plants and promote a range of ecosystem services including plant production, diversity and nutrient cycling.
2. We tested whether AMF have the ability to enhance the resistance and resilience of plant communities under soil moisture deficit (hereby drought) and nitrogen deposition. Grassland microcosms with 11 different plant species were established with and without AMF and exposed to elevated nitrogen levels and to an intermittent period of drought.
3. Drought strongly reduced plant productivity and nitrogen cycling, but had limited effects on plant diversity. Nitrogen enrichment reduced plant diversity and increased nitrogen leaching and N_2O emissions. The presence of AMF enhanced plant productivity, plant diversity, and reduced nitrogen losses. AMF facilitated the resistance of plant productivity and nitrogen cycling to drought and the recovery of the plant community structure back to its pre drought state. Furthermore, AMF also mitigated the adverse effects of nitrogen enrichment on the resistance of multiple ecosystem functions to drought.
4. *Synthesis.* Our work highlights the integral role of AMF for the stability of ecosystem functioning; AMF are not only able to promote resistance to harsh conditions of global change, but also improve resilience by enabling plant

communities to recover. These findings underline AMF's insurance capacity to buffer ecosystems against global change.

KEYWORDS: Plant-soil (below-ground) interactions, global change, arbuscular mycorrhizal fungi, resistance, resilience, ecosystem multifunctionality, drought, nitrogen enrichment

1 INTRODUCTION

Global change is progressing at an unprecedented pace causing more frequent and prolonged periods of drought worldwide (IPCC, 2013, Rockström et al., 2009). Moreover, anthropogenic activities have doubled nitrogen (N) inputs to terrestrial ecosystems via atmospheric deposition (Galloway et al., 2008). Various studies have now shown that these global changes can strongly threaten the temporal stability (Isbell et al., 2009, Tilman & Downing, 1994) and functioning of terrestrial ecosystems (de Vries et al., 2012, Ma et al., 2019, Rillig et al., 2019). The core components of ecosystem stability are well-defined as resistance, *i.e.* the ability of ecosystems to withstand perturbations, and as resilience, *i.e.* the ability of ecosystems to return to their original state after subsequent recovery (Mariotte et al., 2013, Standish et al., 2014). However, there are still critical knowledge deficits as to the role of various ecological interactions in mitigating global change by enhancing the resistance and resilience of ecosystems (Delgado-Baquerizo et al., 2017, Valencia et al., 2015).

A wide range of studies have shown that soil microbial communities are directly impacted by global change (de Vries et al., 2018, Argiroff et al., 2019, Bardgett and Caruso, 2020). Soil microbes are a cornerstone for the maintenance of diverse ecosystem functions such as primary productivity, plant diversity and the above-belowground cycling of carbon and nutrients (Wagg et al., 2019, Tao et al., 2016). Considering their critical roles in ecosystem functioning, soil microbes are also likely key regulators of ecosystem stability, but their importance for ecosystem resistance and resilience against global change perturbations has not yet been clearly elucidated (Delgado-Baquerizo et al., 2017, Yang et al., 2018).

Arbuscular mycorrhizal fungi (AMF) are widely recognized as pivotal

belowground symbiont associating with over 80% of terrestrial plants (Smith & Read, 2008). This plant-fungal mutualism is responsible for promoting productivity and diversity in plant communities (van der Heijden et al., 1998, Wagg et al., 2011), enhancing plant stress resistance (Augé, 2001, Tao et al., 2016) and contribute to the cycling of carbon and nutrients (van der Heijden et al., 2008, Bender et al., 2014, Bowles et al., 2018). Further, this group of plant symbiotic fungi has been shown to be keystone taxa of plant-associated microbiomes (Banerjee et al., 2019). Nonetheless, whether AMF enhance resistance and resilience of the functioning of ecosystems under global change perturbations is still poorly considered within the literature (see Figure S1). In fact, there is no experimental evidence on the role of AMF that explicitly assess the resistance or the resilience of ecosystem functions. Furthermore, the contribution of AMF to ecosystem resistance and resilience is crucial, as AMF themselves are also threatened by global change (Zhang et al., 2016, Garcia & Mendoza, 2008), and their suppression could accelerate the negative effects of global change on the stability of ecosystems. Thus, a next frontier is now to empirically test whether AMF play an important role in the resistance and resilience of ecosystems and in particular how multiple ecosystem functions (e.g. ecosystem multifunctionality, see Giling et al. (2019)) are affected.

Increasing frequency of drought and elevated N deposition, two of the most threating global change factors for terrestrial ecosystems, are leading to severe biodiversity losses, increased greenhouse gas emissions and nutrient leaching, and consequently jeopardising ecosystem stability (Frank et al., 2015, Galloway et al., 2008, Liu et al., 2019, van der Heijden et al., 2008). While the risk of individual factors is well established, it is not fully understood how these factors interact and whether their

simultaneous occurrence exacerbate the harmful effects (Rillig et al., 2019). Here, we simulated a grassland system, one of the major terrestrial ecosystems covering approximately one-fourth of the Earth's land surface (Bartholome & Belward, 2005), to investigate whether AMF enhance the resistance and resilience of diverse ecosystem functions under simultaneously simulated drought and N deposition perturbations. We simulated drought by an intermittent period of soil moisture deficit with and without elevated nitrogen levels. We assessed ecosystem functions by measuring five ecosystem function variables including plant community biomass and diversity, N uptake, N leaching and N_2O emissions. We hypothesised that (1) AMF support ecosystem resistance to drought by enhancing ecosystem functioning under drought condition. We further tested the hypothesis that (2) elevated N deposition compromises ecosystem functioning and thereby reduces ecosystem resistance but also resilience after drought. Finally, we postulate that (3) AMF buffer the adverse effects of elevated N deposition and thereby increase the resistance and resilience of ecosystem functioning against multiple global change factors.

2 MATERIALS AND METHODS

2.1 Plant community and growing conditions

Plant communities were planted into microcosms (PVC tubes of 15 cm diameters and 19.5 cm height, Figure S2) and grown under controlled conditions for 35 weeks in a greenhouse at Agroscope (Zurich, Switzerland) from October 2018 to June 2019. The microcosms had a 16 h at 25°C days and 8 h 17°C nights; relative humidity was 65% at day and 85% at night. Additional light was provided by high-pressure sodium lights when natural light levels reached < 400 W/m^2. Microcosms were equipped with a drain

that could be closed to collect leachate and a removable airtight cap for allowing to seal the headspace for greenhouse gas measurements. Microcosms were filled with a soil mixture (3.49 kg dry weight) of sieved autoclaved soil (121 ℃, 90 min) obtained from calcareous grassland (44° 26′ N, 7° 33′ E; Nenzlingen, Switzerland) and autoclaved quartz sand (1:1.25 w/w). The bottom of each microcosm was covered with a 500 µm PP mesh and a 3 cm layer of autoclaved sand (400 g dry weight) for better drainage.

The plant community consisted of 10 different plant species with a total of 33 plant individuals planted in each microcosm. The number of individuals per plant species is given in Table S1. The composition of the plant community corresponded to the natural relative abundance in typical calcareous grassland in Europe (van der Heijden et al., 2008, van Der Heijden et al., 2006). Seeds of these species were surface-sterilized with 1.25% sodium hypochlorite (diluted bleach) for 10 minutes, thoroughly rinsed with deionized water, and germinated on moist filter paper in sterilized Petri dishes. Seedlings were approximately one week old when planted. The 33 individuals were planted at fixed distances (2.5 cm) from each other according to a predefined design (Figure S2a). Eight different planting designs were used, each design being assigned to one block. Thus, plant individuals belonging to one block had the same neighbour, while plants in different blocks had different neighbours in microcosms. This approach was chosen to reduce potential effects of neighbourhood interactions on the plant community responses (van Der Heijden et al., 2006, van der Heijden et al., 1998). Seedlings that died within the first two weeks of the initial phase of the experiment were replaced to make sure all microcosms contained 33 plant individuals.

2.2 Experimental design

147 The experiment was set up as a complete randomized block and fully crossed
148 design with three factors: 'drought' (ambient/drought), 'N deposition'
149 (ambient/elevated), and 'AMF' (with/without AMF), each factor included two levels
150 yielding in eight treatments, each replicated eight times (eight blocks) for a total of 64
151 microcosms.
152
153 *Mycorrhiza*
154 Half of the microcosms received 100 g inoculum comprised of substrate and root
155 fragments of four AMF single spore cultures (AMF treatment, M), the other half (non-
156 AMF treatment, NM) were inoculated with autoclaved inoculum (121 °C, 90 min). The
157 AMF cultures consisted of the following four species: *Claroideoglomus claroideum*
158 (isolate number, 0913E), *Funneliformis mosseae* (1113B), *Glomus diaphanum* (1013A),
159 and *Rhizoglomus irregularis* (0813B), which all occur commonly in European
160 grassland (Oehl et al., 2010). These four AMF species are deposited in the Swiss
161 Collection of Arbuscular Mycorrhizal Fungi (www.agroscope.ch/saf) and were
162 propagated in the greenhouse on *Plantago lanceolata* with the autoclaved substrate
163 made of 15% grassland soil and 85% sand.
164 Each microcosm received 75 ml microbial wash to correct for the potential
165 differences in microbial communities among different treatments and natural grassland.
166 The microbial wash was prepared by wet-sieving 3 kg soil-inoculum mixtures (2.5 kg
167 of fresh soil collected from the same site and 500 g AMF inoculum) through 10 µm
168 sieve with 6 L deionized water.
169
170 *Nitrogen deposition*

Two levels of N deposition were applied, ambient N deposition (36 kg N/ha, LN) and elevated N deposition (210 kg N/ha, HN). Twenty millilitres of a modified Hoagland nutrient solution (Table S2) were added to the microcosms three times in pre-drought, drought and recovery period, respectively. At the first two applications of each period, we simulated 1 kg N/ha and 10 kg N/ha for ambient N and elevated N deposition, respectively. At the third applications of each period, we applied an N pulse of 10 kg N/ha and 50 kg N/ha for ambient and elevated N deposition, respectively, allowing greenhouse gas measurement (Figure 1a, more details see below). Thus, effective ambient and elevated N deposition level to which the plant communities were exposed is 26 and 160 kg/ha, respectively. Each microcosm received an equivalent of 3.27 kg P/ha during the entire period of experiment.

Drought

All microcosms were maintained at an ambient soil moisture ranging between 24% and 29% (equals to 63% - 75% soil water hold capacity, WHC) for the first 11 weeks as a pre-drought period. The following 11 weeks we imposed drought treatment by reducing soil moisture of the half of microcosms to a level ranging between 14% and 19% (equals to 37% - 50% WHC, D; Singh, 1978, Matias et al., 2018), while the remainder microcosms stayed at ambient conditions (A). Following these 11 weeks drought perturbation soil moisture of all microcosms was brought back to ambient levels and maintained for an additional 11 weeks for a period of recovery (Figure 1a). Different soil moisture ranges were achieved by weighting and watering every or every other day to the corresponding soil moisture level.

195 **2.3 Data collection**

196 At the end of each 11 weeks period aboveground biomass was harvested
197 approximately 2 cm above soil and separated by species, dried at 65 ℃ for 3 days and
198 weighted. Nitrogen uptake by plant community was determined by pooling dried shoots
199 per functional group and ground at 30 Hz in a mixer mill (MM200; Retsch, Haan,
200 Germany) for 2 minutes and N concentrations were determined by C/N automatic
201 analyser (Primacs SNC100-IC) (FAL and Forschungsanstalten, 1996). Several samples
202 did not have enough plant material of separate functional groups and so all plant shoots
203 were pooled.

204 Two soil cores (2 cm diameter, 17 cm deep) were taken from each microcosm
205 during the pre-drought and drought harvests and four soil cores at the recovery harvest.
206 Holes from coring were refilled with the same sterile substrate. Soil cores were sieved
207 to 2 mm to mix thoroughly and collect root samples. Soil was stored in 50 ml tubes at
208 - 20 ℃ for NH_4^+-N and NO_3^--N analyses and roots were stored in 2 ml tubes at -20 ℃
209 for mycorrhizal colonization assessment. Colonisation of roots by AMF was done by
210 clearing roots with 10% KOH and staining with a 5% pen ink-vinegar mixture
211 (Vierheilig et al., 1998), and scored using the line-intersection method (McGonigle et
212 al., 1990) for 100 intercepts per root sample.

213 N_2O fluxes were measured at the time of each biomass harvest by covering the
214 microcosms headspace at a height of 10 cm above soil surface. The headspace was
215 closed for 4 min with gas directly pumped into Picarro G2508 Greenhouse Gas
216 Analyser (Picarro lnc., Santa Clara, CA, USA) (Figure S2b). We first measured
217 background level of greenhouse gas fluxes before adding N pulse. One day later we
218 added nutrients and the N pulse (mentioned above) and measured greenhouse gas fluxes

for 7 days. During first three days gas fluxes were measured three times a day every 8 hours to monitor the emission peak based on Bender et al. (2014). On the following two days it was measured twice every 12 hours and on the last two days once every 24 hours to trace fluxes until they reached the background level. This resulted in 17 gas flux measurements per microcosm. Before each measurement, the microcosms were watered to the soil moisture level of the corresponding treatment with deionized water (see above).

After greenhouse gas flux measurements, the microcosms received an artificial rainfall using a rainfall simulator (2 L, 14 cm in diameter) as described by Knacker et al. (2004; Figure S2c). Rainfall simulators were filled with 1.5 L deionized water that rained into microcosms for approximately 90 min and corresponds to an 80 mm rainfall event. Leachate from each microcosm was collected until the drainage had ceased. Leachate volume was recorded and a subsample of 40 ml of each microcosm was stored at -20 °C to determine mineral N (NH_4^+-N and NO_3^--N) concentrations at the Swiss Federal Research Stations (FAL and Forschungsanstalten, 1996). Leachate NH_4^+ and NO_3^- contents were summed and used as total leachate.

2.4 Statistical analyses

Plant diversity was calculated using Shannon-Wiener diversity index (*H*). Changes of plant community composition were analysed based on Bray-Curtis dissimilarity. In order to summarize overall changes in the performance of plant communities and N cycling we calculated a multifunctionality index by taking the average of Z-transformed values of plant biomass, Shannon-Wiener diversity, total N uptake, cumulative N_2O emission, and leached N as has been done elsewhere (Wagg et al., 2014, Maestre et al.,

2012). Prior to averaging N leached and cumulative N_2O emissions were multiplied by -1 so that a decline from their desirable state corresponds to increasingly negative values.

Resistance which is defined as the ability to withstand perturbation, was determined as the changes in function (e.g. biomass, Shannon-Wiener diversity, total N uptake, cumulative N_2O emission, and leachate N) at the end of drought period as the proportional change between ambient (A) and drought (D) treatments (Baert et al., 2016): $Resistance = (D_{drought} - A_{drought})/A_{drought}$. Thus, when the value is not significantly different from 0, there is no change in functioning between ambient and drought treatments; while negative the values, the weaker the resistance.

Resilience is defined as the ability of ecosystem to recover to its original state following subsequent recovery (ecological resilience; Standish et al., 2014). Similarly, we calculated resilience in functions using data collected at the end of the recovery period as the proportional change between drought and ambient treatments: $Resilience = (D_{recovery} - A_{recovery})/A_{recovery}$. Thus, when the value is not significantly different from 0, there is no change in function between ambient and drought treatment, the ecosystem has fully recovered representing a high resilience.

We also calculated the resistance and resilience in the ecosystem multifunctionality index. All ecosystem function values were brought after Z transformation to an overall mean of 3 and SD of 1 (by addition of three) to avoid a division by values below one, which would cause outliers. Resistance and resilience were calculated as mentioned above for individual functions. In addition, we used Bray-Curtis similarity index of plant species compositions between ambient and drought treatments following drought and following the recovery phase as a measure of

resistance and resilience in plant community structure (de Vries et al., 2018, Baert et al., 2016).

Three-way factorial ANOVA was performed to test the main and interactive effects of drought (D), AMF (M) and elevated N deposition (N) on biomass, Shannon-Wiener diversity, total N uptake, cumulative N_2O emission, leachate N and ecosystem multifunctionality at pre-drought, drought, and recovery period, respectively (Table S3). Secondly, two-way ANOVA were used to test the main and interactive effects of AMF (M) and elevated N deposition (N) on the proportional changes to drought of biomass, Shannon-Wiener diversity, total N uptake, cumulative N_2O emission, leachate N and ecosystem multifunctionality at drought and recovery period, indicating ecosystem resistance and resilience respectively (Table S6). Bray-Curtis similarity index data were analysed by using repeated measures ANOVA (Table S5). Finally, t-tests were used to compare the proportional changes (resistance and resilience) of biomass, Shannon-Wiener diversity, total N uptake, cumulative N_2O emission, leachate N and ecosystem multifunctionality in different treatments (NM-LN, NM-HN, M-LN, M-HN) to 0 at drought and recovery period, respectively (Table S4).

All statistical analyses were conducted using SPSS 17.0 (SPSS Inc, Chicago, IL, USA). All figures were made using SigmaPlot 10.0 (Systat Software Inc., Chicago, IL, USA).

3 RESULTS

3.1 Impact of drought, elevated N deposition and AMF on ecosystem functions during pre-drought, drought and recovery period

AM fungal colonisation in the presence AMF across the three periods were more

than 85%, while in the absence of AMF were no more than 3% (Figure S3). During the pre-drought period, AMF strongly increased plant community biomass, Shannon-Wiener diversity, total N uptake, and reduced the peak of N_2O flux, cumulative N_2O emission and leachate N (Figure S4). Elevated N deposition had no effects on plant community biomass, Shannon-Wiener diversity and total N uptake, but strongly increased the N_2O flux peak, cumulative N_2O emission and leachate N (Figure S4j and S4m). There were clear interactive effects of AMF and elevated N deposition on cumulative N_2O emission and leachate N (Figure S4). Immediately after fertilisation and watering, N_2O emission curves clearly increased with elevated N deposition (50 kg N/ha; Figure 2) in the absence of AMF, but did not change with the ambient N deposition in all treatments (10 kg N/ha; Figure S5). With the high N pulse, after this initial phase, the peak of N_2O flux was lower and recovered faster to the background levels in the presence of AMF compared to the absence of AMF at the end of pre-drought (Figure 2a).

During the drought period, drought significantly decreased plant community biomass, total N uptake, peak of N_2O flux, and cumulative N_2O emission (Figure 1b; Figure S4). Drought had no effects on Shannon-Wiener diversity (Figure 1b; Table S3), but leachate N obtained after simulated rainfall at the end of drought period increased strongly (Figure 1b; Figure S4). The presence of AMF strongly increased plant community biomass, Shannon-Wiener diversity, and total N uptake (Figure 1b; Figure S4). The presence of AMF caused an 87.7% decrease of leachate N (Figure 1b, Figure S4n) and strongly reduced the peak of N_2O emission under drought conditions (Figure 2b). It is notable that there were interactive effects of drought and AMF on cumulative N_2O emission. AMF strongly reduced cumulative N_2O emission under drought water

conditions, while had no effect on cumulative N_2O emission under ambient water conditions (Figure 1b; Figure S4). Interestingly, effects of AMF on N uptake, N_2O emission and N leaching was much stronger than the effects of elevated N deposition. Presence of AMF highly increased total N uptake and reduced N leaching, while elevated N deposition showed no clear effect on any of the assessed N cycle features, except N_2O emission.

During the recovery period, when soil moisture of drought treatment recovered to ambient level, there were no significant differences in biomass, cumulative N_2O emissions and leachate N between ambient and drought treatment (Figure 1b; Table S3). While Shannon-Wiener diversity and total N uptake of drought treatment were higher than that of the ambient treatment, especially in the presence of AMF (Figure 1b; Figure S4). AMF and elevated N deposition strongly increased plant community biomass, Shannon-Wiener diversity and total N uptake (Figure 1b; Figure S4). While AMF decreased cumulative N_2O emission (a reduction with 49.1%) and leachate N (a reduction with 87.0%); elevated N deposition increased cumulative N_2O emission (16.8 times) and leachate N (34.71 times) (Figure 1b, Figure S4l and S4o). There were strong interactive effects of AMF and elevated N deposition on total N uptake, cumulative N_2O emission and leachate N. In particular, AMF mitigated cumulative N_2O emission and leachate N more clearly at elevated N deposition than that at ambient N deposition (Figure S4l and S4o). It is notable that N_2O flux and cumulative N_2O emission were much higher than that at the pre-drought and drought period, and even the peak of N_2O emission could be detected at the low N pulse but only in the absence of AMF (Figure S5c).

AMF increased all ecosystem functions on average (multifunctionality) during all

three pre-drought, drought, and recovery periods (Figure 4a and 4b; Figure S6). In contrast, elevated N deposition strongly decreased ecosystem multifunctionality. During the drought period, drought treatment significantly decreased ecosystem multifunctionality; while during the recovery period, there were no differences between drought and ambient treatments (Figure 4a and 4b). Moreover, there was a significant interactive effect of AMF and elevated N deposition on ecosystem multifunctionality; ecosystem multifunctionality was significantly reduced by elevated N deposition in the absence of AMF, while was not reduced in the presence of AMF across the three periods (Figure 4; Figure S6).

3.2 AMF enhance resistance and resilience of ecosystem functions to drought under elevated N deposition

We determined drought resistance and resilience of multiple ecosystem functions by assessing proportional changes between ambient and drought treatments during the drought (resistance) and recovery period (resilience).

During the drought period, drought resistance of plant community biomass, total N uptake, and leachate N (reverse conversion) across all treatments were significantly lower than 0 (Figure 3; Table S4), while drought resistance of cumulative N_2O emission were only significantly lower than 0 in presence of AMF (Figure 3e; Table S4). Bray-Curtis similarity index was significantly lower compared to the pre-drought period (Figure 3c; Table S5), but there were no differences of Shannon-Wiener diversity between drought and ambient treatments (Figure 3b; Table S4). Elevated N deposition strongly decreased drought resistance of cumulative N_2O emission and leachate N (Figure 3), while elevated N deposition had no effects on the drought resistance of plant

community biomass, Shannon-Wiener diversity, Bray-Curtis similarity index and total N uptake (Figure 3). There were clear interactive effects of AMF and elevated N deposition on the drought resistance of total N uptake, cumulative N_2O emission and leachate N (Table S6). AMF increased the drought resistance of plant community biomass, cumulative N_2O emission, and leachate N, especially at the elevated N deposition (Figure 3); while AMF had no effects on the drought resistance of Shannon-Wiener diversity and total N uptake (Figure 3).

During the recovery period, based on t-tests, drought resilience of Shannon-Wiener diversity in the presence of AMF were significantly higher than 0, and the drought resilience of total N uptake was higher than 0 only in the presence of AMF at elevated N deposition (Figure 3; Table S4). While drought resilience of plant community biomass, cumulative N_2O emission and leachate N were not different comparing with 0 (Figure 3; Table S4). Notably, only the Bray-Curtis similarity index in the presence of AMF was not significantly different compared to the pre-drought period (Figure 3c; Table S5).

Finally, we calculated ecosystem multifunctionality and subsequently assessed the effects of AMF and elevated N deposition on the resistance and resilience of ecosystem multifunctionality to drought. During the drought period, drought resistance of ecosystem multifunctionality were significantly lower than 0, except in the presence of AMF at elevated N deposition (Figure 4c; Table S4). Importantly, there were interactive effects of AMF and elevated N deposition on the drought resistance of ecosystem multifunctionality, AMF increased drought resistance of ecosystem multifunctionality, especially under elevated N deposition (Figure 4c). In contrast, during the recovery period, only the treatment combining presence of AMF and elevated N deposition

exceeded the control value (Figure 4c; Table S4).

4 DISCUSSION

Ecosystem functioning and stability are threatened by simultaneous changes of multiple biotic and abiotic factors such as increasing frequency of extreme drought events, elevated atmospheric N deposition and biodiversity loss (Hoover et al., 2014, Rockström et al., 2009, Rillig et al., 2019). By integrating major ecological functions such as primary production, plant diversity and nutrient cycling, our work provides compelling experimental evidence that AMF not only facilitate multiple ecosystem processes (multifunctionality), but also increase their resistance to drought and the ability to recover from drought, particularly under simultaneous elevated N deposition.

4.1 AMF enhance resistance and resilience of ecosystem functions to drought

Our work emphasises the integral role of AMF for the stability of ecosystem multifunctionality, while earlier studies had similarly indicated the importance of the wider soil microbiome (Delgado-Baquerizo et al., 2017, Schäfer et al., 2019). These studies focus either on the stability of a single ecosystem function, in particular primary production (Hoover et al., 2014, Schäfer et al., 2019), or addressed multifunctionality resistance to drought by using systems with a single plant species or even without plants (Bennett et al., 2019, Delgado-Baquerizo et al., 2017, de Vries et al., 2012). We extend these observations to a more diverse model by simulating a typical European grassland and focus on the role of AMF in ecosystem multifunctionality stability by assessing plant community features and N cycling functions.

It is not surprising that AMF were able to increase drought resistance of the

experimental grassland, as they are known to be particularly beneficial under drought conditions (Aroca et al., 2007, Augé, 2001, Bowles et al., 2018, Mariotte et al., 2017). For instance, AMF facilitate hydraulic lift and thereby increase plants drought resistance (Egerton-Warburton et al., 2007, Singh et al., 2019, Aroca et al., 2007). In addition, mycorrhizal symbiosis are able to improve overall plants nutritional status, which in turn can improve stomatal conductance, and photosynthetic efficiency (Augé et al., 2007, Ruiz-Sánchez et al., 2010), broadly regarded as important drought resistance mechanisms (Subramanian et al., 2006, Omirou et al., 2013, Mariotte et al., 2017).

While AMF showed a pivotal role in the resistance, less impact has been revealed on drought resilience of the experimental grassland system. However, AMF facilitated compensatory recovery of plant diversity, community structure and ecosystem multifunctionality. The importance of AMF for rapid recovery of plant growth after drought has been reported earlier (Aroca et al., 2007, Subramanian et al., 1997). We are here able to extend the importance of AMF for the resilience of plant community composition and N cycling. A recent review elaborated that compositional recovery of plant communities is an important driver for high recovery of ecosystem multifunctionality (Hillebrand & Kunze, 2020). Therefore, the observed effect of AMF on the resilience of multifunctionality may be strongly intertwined with its direct effects on plant community structure recovery.

The plants maintained mycorrhizal symbiosis throughout all periods of the experiment, as indicated by the stable extent of root colonisation even during and after drought stress. Such an unconditional maintenance of the symbiosis even under drought stress has also been shown in earlier studies (Augé, 2001, Jacobson, 1997). It may be

only this unconditional maintenance of the symbiosis that has enabled AMF to enhance the resistance and resilience of ecosystems to drought. Our findings demonstrate the important role of AMF in ecosystem resilience, and give a clear indication of AMF's insurance capacity for ecosystems suffering from the next extreme climate changes.

4.2 AMF buffer adverse effects of elevated N deposition on resistance and resilience of ecosystem functions to drought

Elevated N deposition reduced multifunctionality of the experimental grassland and in particular its resistance to drought. However, AMF were able to mitigate these adverse effects of the two simultaneously occurring global change factors. Our results are in line with a previous study showing that simultaneous N enrichment magnified ecosystem sensitivity to drought by reducing resistance of plant community biomass to drought (Bharath et al., 2020). Drought resistance of multifunctionality was greatly impacted by interactive effects between AMF and N enrichment. AMF increased the resistance mainly under elevated N deposition. Under ambient N deposition, N input may have been too low to reveal a similar impact of AMF, as for instance no clear N_2O emission peak could be observed. Under elevated N deposition, AMF increased resistance of multifunctionality to drought by increasing the resistance of total N uptake and N leaching. These results suggested that roles of AMF in nutrient cycle could be an important mechanism underlying the observed AMF-driven resistance of ecosystem multifunctionality to drought (Ruiz-Sánchez et al., 2010, Augé, 2001).

Similarly, AMF-driven resilience of multifunctionality could be only revealed under elevated N deposition. One could speculate that additional N input under elevated N deposition relaxed the competition among plants and AMF for the scarce resource N,

459 which may have allowed increasing benefits of AMF to the grassland system (Scheublin
460 et al., 2007, Johnson, 2010). The more evident effect of AMF on resilience of
461 multifunctionality to drought at elevated N deposition could thus indicate the
462 importance of AMF for ecosystem stability may even increase under simultaneous
463 changes of multiple factors.

464 Finally, the severe impacts observed during drought period vanished during
465 recovery period and ecosystem functioning could quickly recover to the level of
466 ambient conditions after rewetting. This is in accordance with the conclusion of
467 Hillebrand and Kunze (2020) that more than 80% of the studies report complete
468 restoration of ecosystem functions in different ecosystems after pulse disturbances such
469 as drought. Thus, a more dynamic monitoring with several time points after rewetting
470 would improve our understanding of the recovery process. Moreover, our results derive
471 from an experimental grassland system grown under controlled conditions; future work
472 should be carried out under natural conditions and across different ecosystems to assess
473 the general validity of our results.

474

475 **5 CONCLUSIONS**

476 Together, our findings identify a novel role of AMF in maintaining and stabilising
477 ecosystem functioning in face of global change. Our results provide compelling
478 evidence that AMF not only increase the resistance of multiple ecosystem functions to
479 drought, but also mitigate adverse effects of simultaneously elevated N deposition. We
480 further observed AMF-driven compensatory recovery of plant community structure and
481 ecosystem multifunctionality. Overall, our findings suggest that AMF not only directly
482 support diverse ecosystem functions, but also play critical roles as key soil microbial

taxa in sustaining stability of ecosystem multifunctionality. This is an indication that the loss of biodiversity or of certain functional groups could amplify the adverse effects of simultaneously acting factors of global change. In the future, we need more realistic climate change experiments, which consider the role of soil microbes, such as AMF, to better predict responses of ecosystems to global change using the concept of stability in this ongoing global change world (Korell et al., 2019).

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AUTHORS' CONTRIBUTIONS

Y.J., M.G.A.v.d.H and F.W. conceived and designed the study with suggestions from C.W. and G.F. Y.J. performed the experiment, collected, and conceptualized the data. Y.J. and F.W. wrote the first draft of the manuscript with substantial contribution of M.G.A.v.d.H and C.W. All authors edited the manuscript.

DATA AVAILABILITY STATEMENT

Data are available at the Dryad Digital Repository: https://doi.org/10.5061/dryad.q573n5tgc (Jia et al., 2020)

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754

Figure captions

FIGURE 1 Patterns of soil moisture and N deposition at pre-drought, drought and recovery periods (a). Heat map illustrating effects of experimental treatments on biomass, Shannon-Wiener diversity, total N uptake, cumulative N_2O emission and leachate N during the drought and recovery periods (b). The experimental treatments included ambient and elevated N deposition (LN, HN), non-AMF and AMF status (NM, M) and ambient or drought water regimes (A, D). The values of biomass, Shannon-Wiener diversity, total N uptake, cumulative N_2O emission and leachate N from each period were standardized by Z transformation (see Materials and Methods, please note: the larger values of cumulative N_2O emission and leachate N, the more negative). Green colour indicates positive value (>0, increase), red colour indicates negative value (<0, decrease). Different lowercase letters represent significant difference between different treatments with LN at $p<0.05$ level; Different uppercase letters represent significant difference between different treatments with HN at $p<0.05$ level. Original data see Figure S4.

FIGURE 2 Impact of drought and AMF treatment on N_2O flux after a fertilization pulse corresponding to 50 kg N/ha (elevated N deposition) at the end of pre-drought (a), drought (b) and recovery period (c). Blue line represents no AMF treatment (NM), red line represents AMF treatment (M), solid line represents ambient watering treatment (A), and dash line represents drought treatment (D).

FIGURE 3 Proportional changes in plant community biomass (a), Shannon-Wiener diversity (b), Bray-Curtis similarity index (c), total N uptake (d), cumulative N_2O

779 emission (e), and leachate N (f) to drought as affected by AMF and N deposition
780 treatments during drought and recovery. Blue line represents no AMF treatment (NM),
781 red line represents AMF treatment (M), light colour represents ambient N deposition
782 (LN), bright colour represents elevated N deposition (HN). Proportional changes in
783 drought period represent drought resistance, while in recovery period represent drought
784 resilience. Blue and red asterisk represents the significant difference than 0 based on t-
785 test. Different lowercase letters represent significant difference between different
786 periods in the presence of AMF at $p<0.05$ level; Different uppercase letters represent
787 significant difference between different periods in the absence of AMF at $p<0.05$ level
788 (Figure 4c). Results of two-way ANOVA analysis of the effects of M, N and their
789 interactions on the proportional changes of ecosystem functions during drought period
790 were shown in the figure; but the two-way ANOVA analysis of all ecosystem functions
791 were not significant during recovery period, so data was not shown in the figure. Error
792 bar indicates ± SE. Note: the values of leachate N were multiplied by -1. *, $p<0.05$; **,
793 $p<0.01$; ***, $p<0.001$; NS, non-significant
794
795 **FIGURE 4** Impact of drought, AMF and elevated N deposition on ecosystem
796 multifunctionality (EMF) at drought (a) and recovery (b); the proportional changes of
797 EMF in presence and absence of AMF and elevated N deposition at drought and
798 recovery periods (c); and results of three-way ANOVA analysis of drought (D), AMF
799 (M), elevated N deposition (N) and their interactive effects on EMF during drought and
800 recovery period (d). Blue colour represents no AMF treatment (NM), red colour
801 represents AMF treatment (M), light colour represents ambient N deposition (LN),
802 bright colour represents elevated N deposition (HN), solid circle represents ambient

803 treatment (A), and hollow circle represents drought treatment (D). Different lowercase
804 letters represent significant difference between different treatments with LN at $p<0.05$
805 level; Different uppercase letters represent significant difference between different
806 treatments with HN at $p<0.05$ level. Red asterisk represents the significant difference
807 of M-HN than 0 based on t-test (Table S4). Results of two-way ANOVA analysis of the
808 effects of M, N and their interactions on the proportional changes of EMF during
809 drought period were shown in the figure; but the two-way ANOVA analysis during
810 recovery period were not significant, so data was not shown in the figure (c). Error bar
811 indicates ± SE. Note: proportional changes in drought period represent drought
812 resistance, while in recovery period represent drought resilience. *, p<0.05; **, p<0.01;
813 ***, p<0.001; NS, non-significant
814

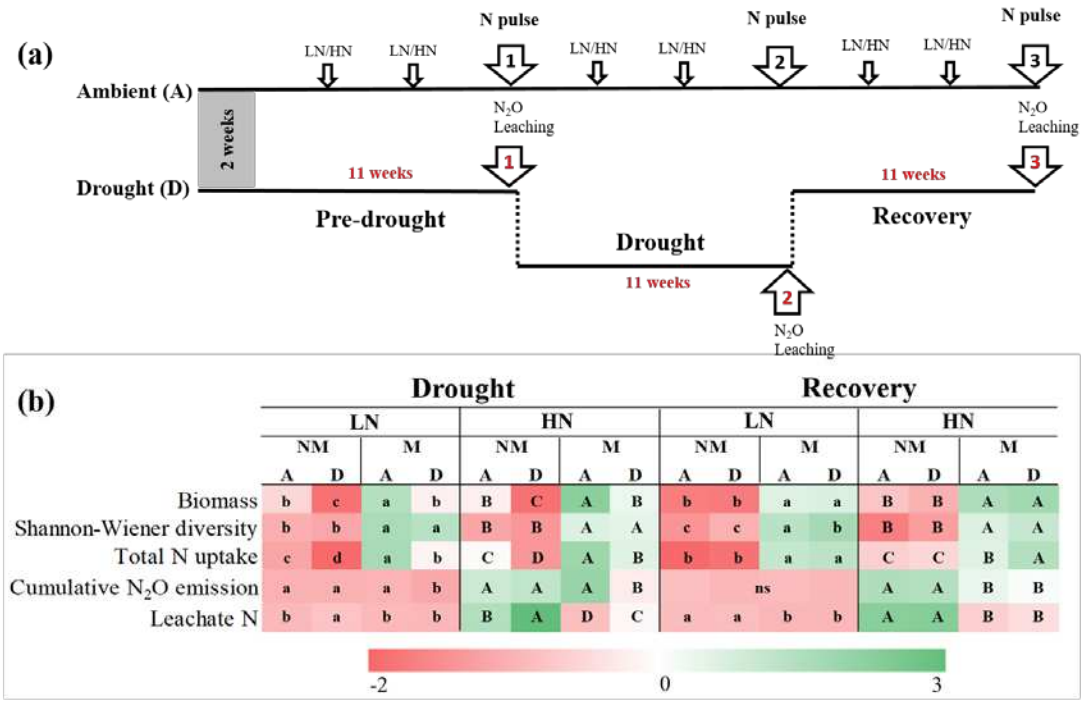

(a)
Ambient (A)
Drought (D)
2 weeks
LN/HN
N pulse
1
2
3
N2O Leaching
11 weeks
Pre-drought
Drought
Recovery
(b)
Drought
Recovery
LN
HN
NM
M
A
D
Biomass
Shannon-Wiener diversity
Total N uptake
Cumulative N2O emission
Leachate N
ns
-2
0
3

FIGURE 1

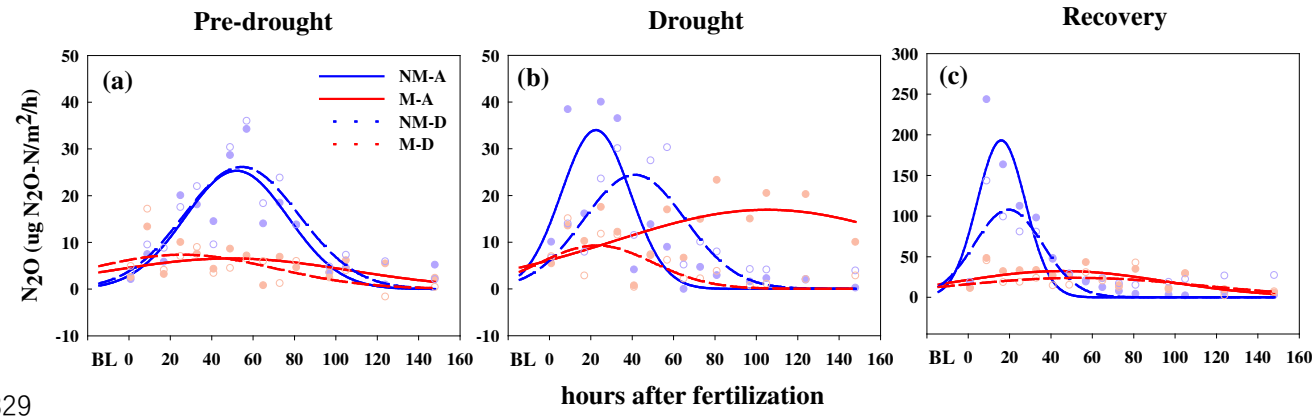

Pre-drought
Drought
Recovery
(a)
(b)
(c)
NM-A
M-A
NM-D
M-D
N2O (ug N2O-N/m2/h)
BL 0 20 40 60 80 100 120 140 160
hours after fertilization

FIGURE 2

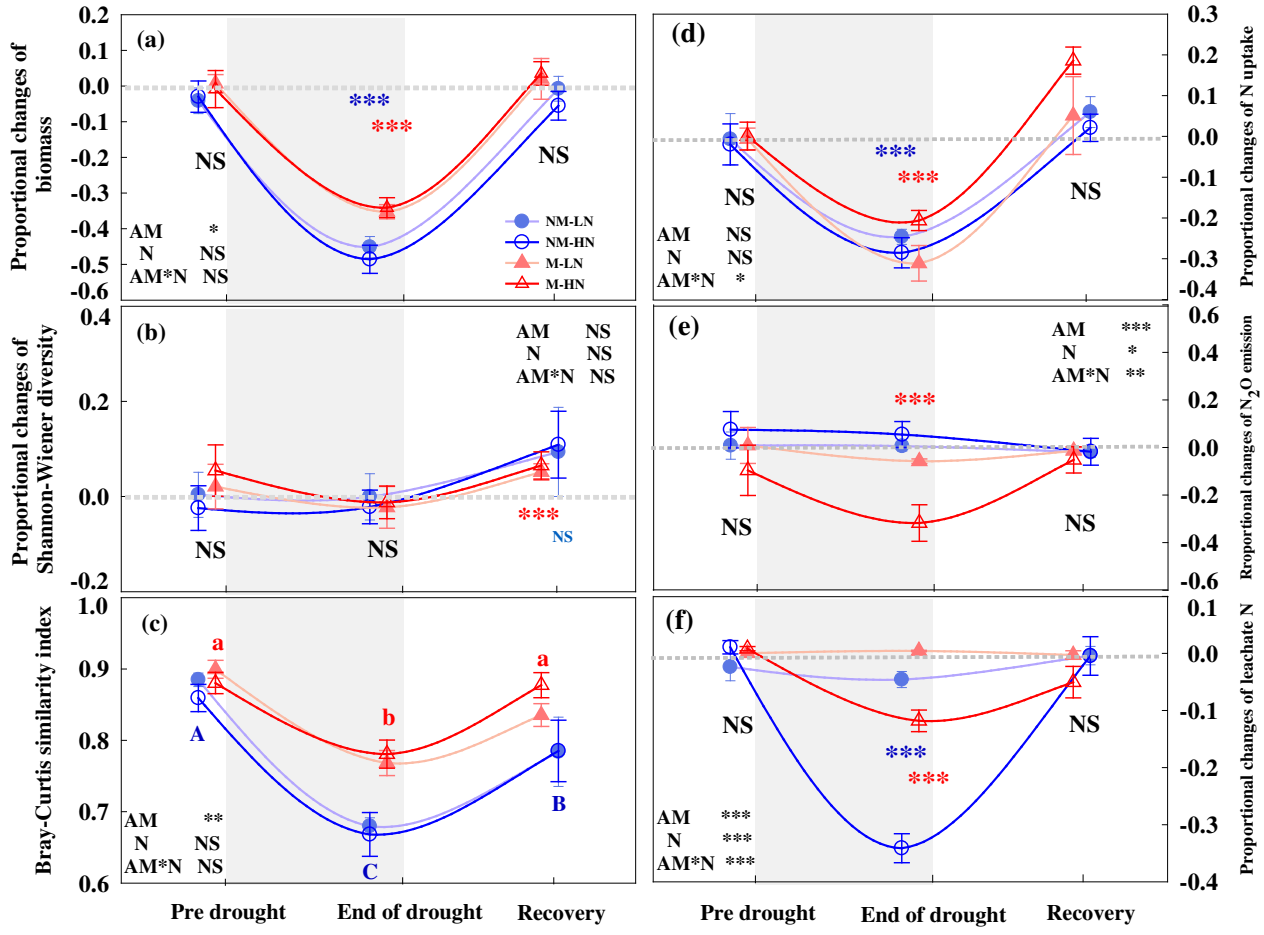
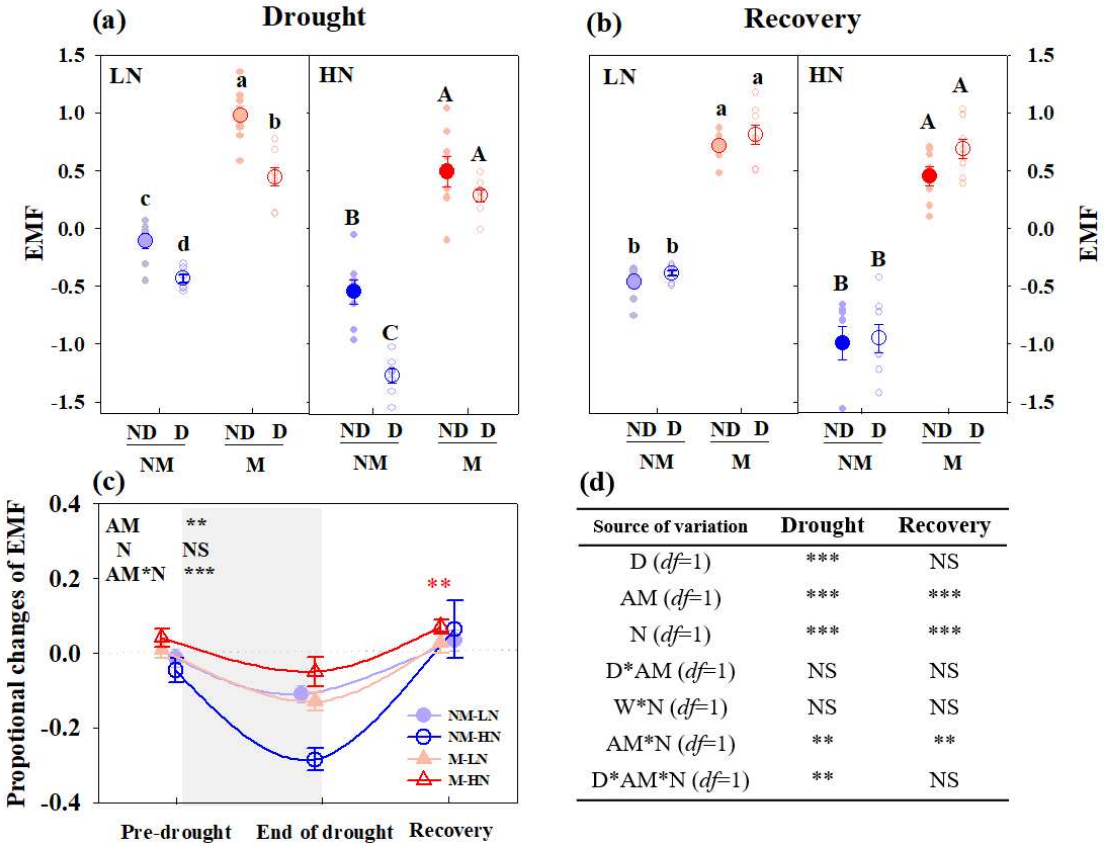

(a)
(b)
(c)
(d)
(e)
(f)
Proportional changes of biomass
Proportional changes of Shannon-Wiener diversity
Bray-Curtis similarity index
Proportional changes of N uptake
Proportional changes of N_2O emission
Proportional changes of leachate N
NM-LN
NM-HN
M-LN
M-HN
AM * N NS AM*N NS
AM NS N NS AM*N NS
AM ** N NS AM*N NS
AM NS N NS AM*N *
AM *** N * AM*N **
AM *** N *** AM*N ***
Pre drought
End of drought
Recovery

FIGURE 3



Source of variation	Drought	Recovery
D (*df*=1)	***	NS
AM (*df*=1)	***	***
N (*df*=1)	***	***
D*AM (*df*=1)	NS	NS
W*N (*df*=1)	NS	NS
AM*N (*df*=1)	**	**
D*AM*N (*df*=1)	**	NS

FIGURE 4